

Fully automated high-throughput chromatin immunoprecipitation for ChIP-seq: Identifying ChIP-quality p300 monoclonal antibodies.

Supplementary information

Supplementary Methods

DNA sequencing library construction for Illumina platform

DNA fragments recovered from ChIPs or reverse cross-linked chromatin are repaired, ligated to adapters, size selected and PCR-amplified to make the library for sequencing. Illumina DNA Library Construction Kit reagents are substituted in this protocol with reagents from NEB and Finnzymes, except for the adapter oligo mix and the PCR primers, which can be ordered from Illumina. We currently use paired-end adapters for library construction, even though we sequence our ChIP libraries with a single-end sequencing run. DNA in EB can be stored in the freezer at any step below.

After reverse crosslinking, DNA fragments recovered from ChIP or from control chromatin samples are repaired, ligated to Illumina Paired-End adaptor sequences and PCR-amplified to complete the adaptor sequences and introduce a 7-base DNA barcode in the i7 position. The barcode allows for mixing of multiple samples per flowcell lane. Control libraries are prepared from 500 ng of DNA from reverse crosslinked sonicated chromatin. ChIP library starting amounts varied by ChIP, with a median of 7.5 ng. Paired-end adaptors are used for library construction, even though libraries are sequenced with a single-end 50 bp sequencing run. DNA in EB can be stored at -20°C at any step below. Updates and modification to this protocol are made available at <http://research.hudsonalpha.org/Myers/>.

1. End Repair

- (a) Mix in a PCR tube on ice:

5.0 μL 10X T4 DNA ligase buffer (supplied with NEB M0202)
0.5 μL 10 mM dNTP mix (NEB N0447)
41.5 μL recovered DNA fragments
1.0 μL T4 DNA polymerase (NEB M0203)
1.0 μL T4 Polynucleotide Kinase (NEB M0201)
1.0 μL Klenow DNA polymerase (NEB M0210)

Total volume = 50 μL

When processing more than one sample, a master mix that includes all reagents except for the recovered DNA fragments can be prepared.

- (b) Spin down briefly in a mini centrifuge and incubate at 20°C in a thermal cycler for 30 minutes.
(c) Purify on a QIAquick PCR cleanup column (QIAquick PCR Purification Kit, Qiagen 28104) per the QIAquick protocol and elute with 32 μL EB warmed to 55°C . Allow EB to soak the filter of the column for 1 minute before centrifuging for 1 minute at the final step.

2. dA Addition

- (a) Mix in a PCR tube on ice:

32 μL end-repaired DNA fragments
10 μL 1mM dATP (NEB N0440S diluted to 1mM)
5 μL 10X NEBuffer2 (supplied with NEB M0212)
3 μL Klenow fragment (3' to 5' exo-; NEB M0212)

Total volume = 50 μL

- (b) Spin down briefly in a mini centrifuge and incubate at 37°C in a thermal cycler for 30 minutes.
(c) Purify on a QIAquick PCR cleanup column (as in Step 1 above) and elute with 42 μL EB warmed to 55°C . Allow EB to soak the filter of the column for 1 minute before centrifuging for 1 minute at the final step.

3. Adapter ligation

- (a) Mix in a PCR tube on ice:
 - 42 μ L DNA recovered from dA addition (Step 2)
 - 5 μ L T4 DNA Ligase buffer (supplied with NEB M0202)
 - 0.5 μ L Paired-end adapter oligo mix
 - 0.5 μ L ddH₂O
 - 2 μ L T4 DNA ligase (NEB M0202)Total volume = 50 μ L
- (b) Spin down briefly in a mini centrifuge and incubate at 20 °C in a thermal cycler for 15 minutes.
- (c) Purify DNA fragments with Agencourt Ampure XP beads (Beckman-Coulter), following manufacturers recommendations (1.8 μ L AMPure XP per 1.0 μ L of total volume in step 3a). Elute with 32 μ L EB.

Agencourt Ampure XP beads use solid phase reversible immobilization (SPRI) technology to recover dsDNA greater than 100bp. This step is carried out instead of gel fragment size selection since it serves to exclude adapters that were not ligated to DNA (mimicking the lower threshold of gel size selection). An extension time of 30 seconds during PCR amplification (step 4 below) ensures that the fragments sequenced are not too long (mimicking the upper threshold of gel size selection). This size selection method consistently produces final DNA library fragments that range from ~100 to 400 bp, as determined by BioAnalysis.

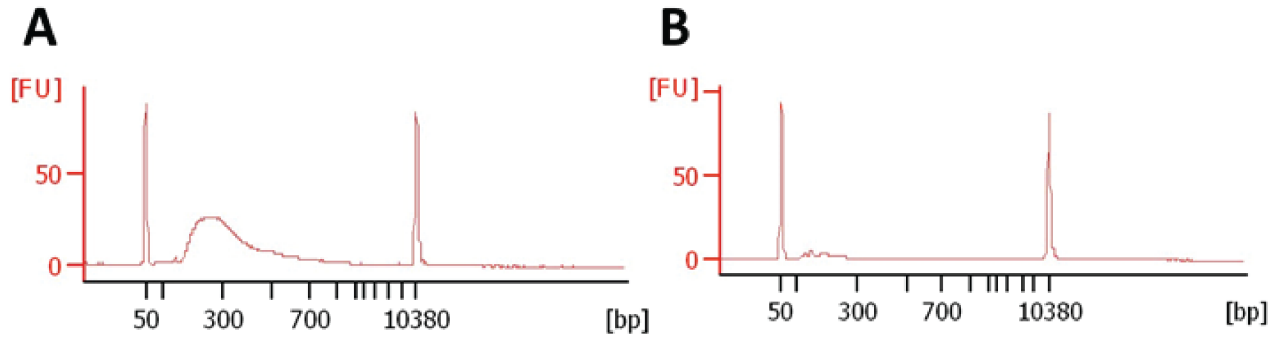
4. Library Amplification by PCR

- (a) Mix in a PCR tube on ice:
 - 32 μ L DNA fragments
 - 36 μ L Phusion DNA Polymerase Mix (Finnzymes, F531)
 - 4.0 μ L PCR primer mixTotal volume = 72 μ L
- (b) Spin down briefly in a mini centrifuge.
- (c) Amplify in thermal cycler using the following protocol:
 - 98 °C for 30 sec
 - 15 cycles of:
 - 98 °C for 10 sec
 - 65 °C for 30 sec
 - 72 °C for 30 sec
 - 72 °C for 5 min
 - 4 °C hold
- (d) Purify the final libraries with Agencourt Ampure XP beads, following the manufacturer's recommendations (1.8 μ L AMPure XP per 1.0 μ L of total volume in step 4a). Elute in 32 μ L EB.

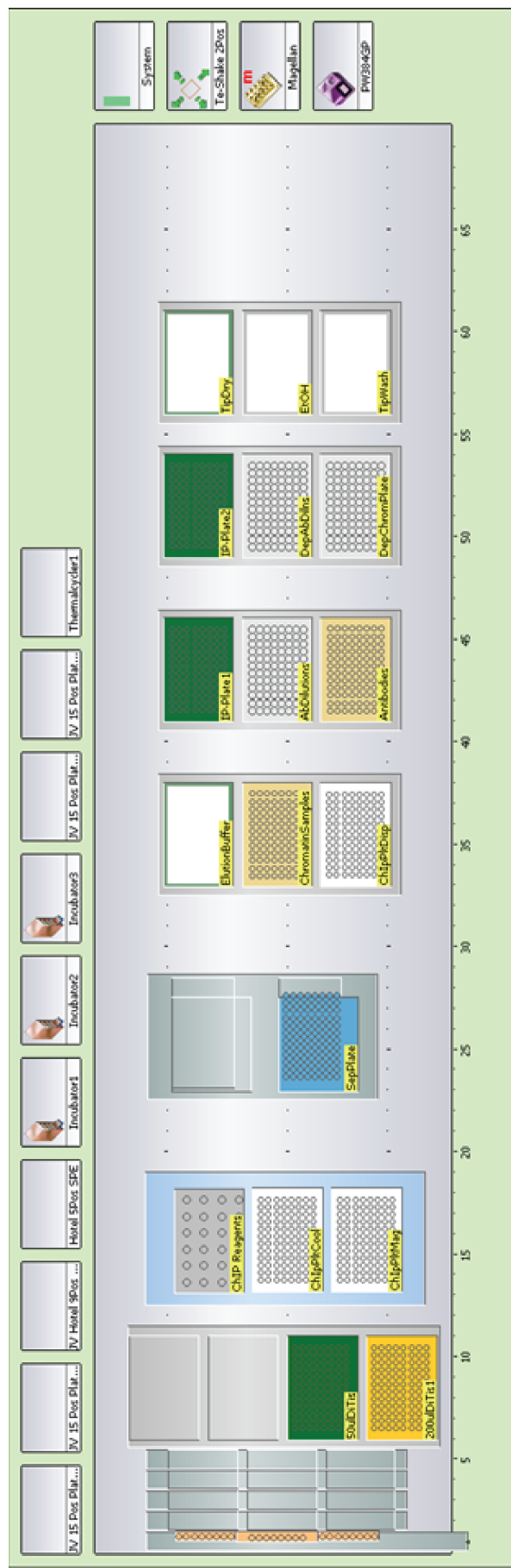
5. Library QC and sequencing

- (a) Quantify final library amounts with a Qubit fluorometer and a dsDNA HS (high sensitivity) Assay Kit (Life Sciences Q32854). Final library amounts typically range from ~50 to 2000 ng, with a median of 1075 ng. There is no minimum requirement for sequencing, but we do require an enrichment of fragments at ~150 to 350 bp as determined by BioAnalysis (see step b below).
- (b) Analyze library size distribution on a BioAnalyzer with an Agilent DNA 7500 Kit (Agilent 5067-1506). A good ChIP-seq library will have an enrichment of fragments in the 150-350 bp range (Supplementary Figure 1A). A library that shows no enrichment of fragments in the 150-350 bp range (Supplementary Figure 1B) indicated either a failed ChIP experiment or failed library construction. These libraries are not sequenced and the experiment is repeated.

Supplementary Figures



Supplementary Figure 1: BioAnalyzer electrophoregrams of a successful (A) and failed (B) ChIP-seq library. A Library that meets the criteria in Steps 5a and 5b is now ready for sequencing on the Illumina HiSeq platform per Illumina's recommendations. In our workflow, the concentration of adaptor-ligated molecules is determined with a KAPA Library Quantification Kit. Then, the concentration and estimated average fragment size (from BioAnalysis) are used to make a pool of libraries with equimolar amounts. Finally, the pool is loaded onto the flowcell for cluster generation at a final concentration of 16.5 pmole. Eight libraries are pooled per lane to generate 20-25 million aligned 50 bp single-end reads per ChIP-seq library.



Supplementary Figure 2: Tecan Freedom Evo 200 configuration for R-ChIP method:

- Liquid handler model:**
Freedom Evo 200
- Robot Arms:**
- 1) Integrated 8-channel liquid handling arm (LiHa, left position) with additional 8-channel large volume dispense technology (Te-Fill technology);
 - 2) 96-Channel multi-channel arm (MCA, middle position);
 - 3) Robotic manipulator arm, standard z-rail (RoMa, right position)

Labware carriers:

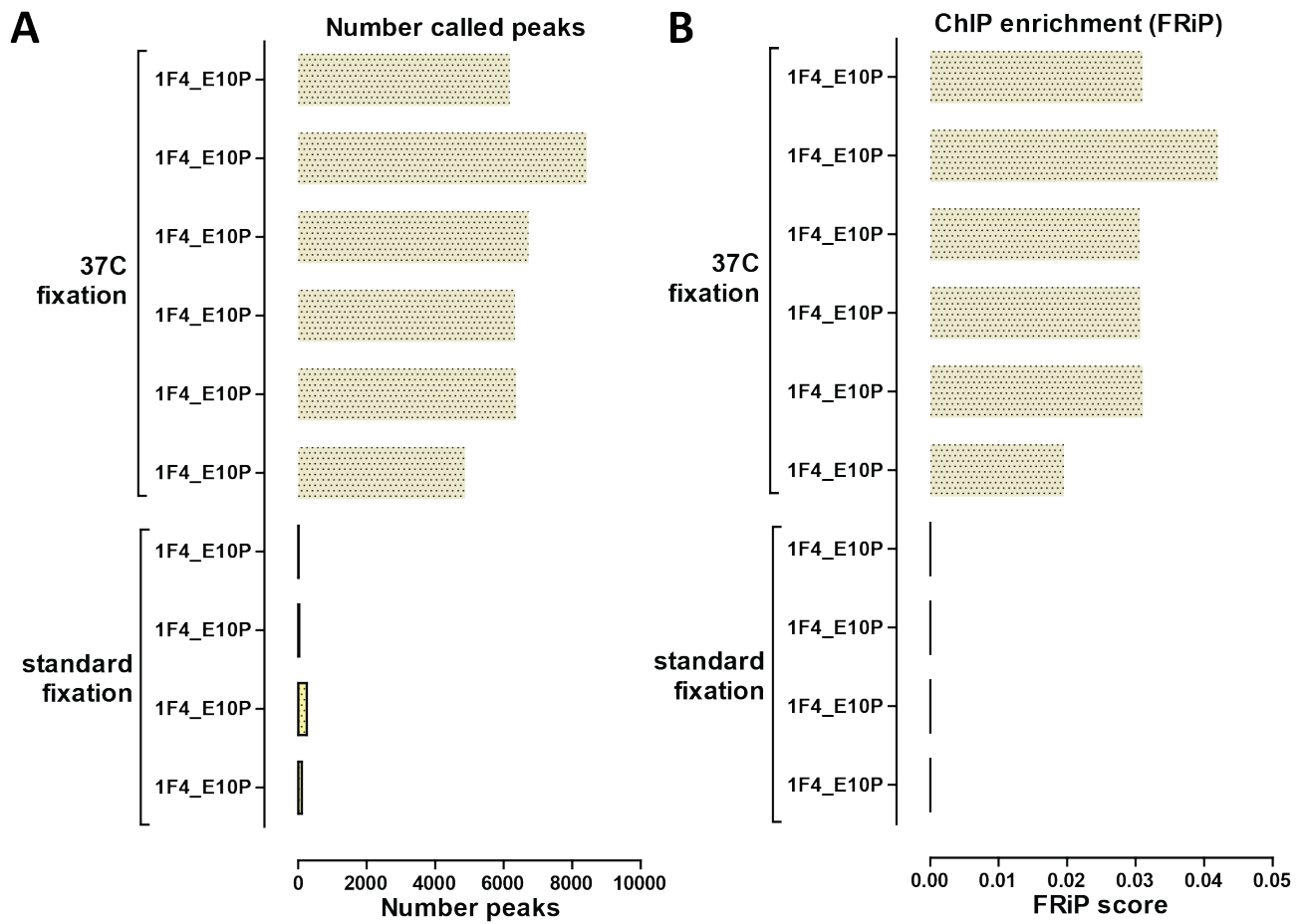
- 1) Disposable tip carrier, for MCA tips, 4-Position;
- 2) Microplate carrier, 3-position, landscape, temperature controlled through external recirculator;
- 3) Microplate carrier, 3-Position, Landscape, Low Profile

Device carriers:

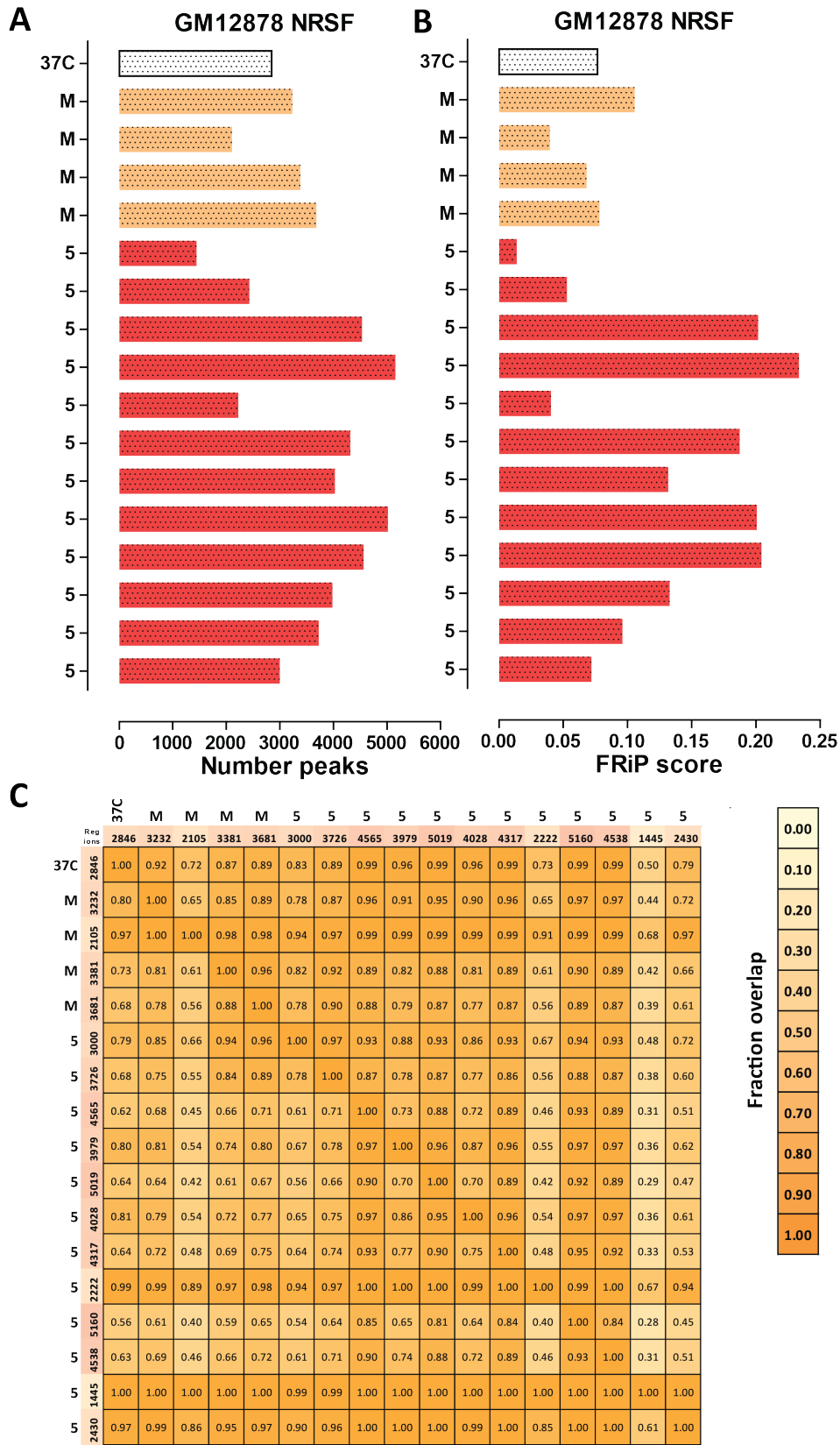
- 1) Te-Shake microplate shaker unit with microplate Nest with hold down;
 - 2) Integrated PCR machine (Moto Alpha 96-well Unit, BIORAD) powered by Remote alpha dock and controlled by PTC200 unit.
- Magnet plate:**
Agencourt SPRIPlate, Ring Super Magnet Plate 96-well (Beckman-Coulter)
- Custom accessories:**
- 1) Peristaltic pump driven MCA tip-wash station (Water, Ethanol-baths, paper blot station for tip drying)
 - 2) Quick connect buffer bottles for Te-Fill dispenser

		H3k4me1	H3k27ac	DNase	Fraction overlap
		134,589	56,069	117,684	
1F4_E10p	4870	0.97	0.91	0.94	
5F7_C9p	2868	0.97	0.92	0.96	
7H5_F2p	1524	0.98	0.94	0.96	
1F4_E10p-Rep2	6374	0.95	0.88	0.89	
sc584-Rep1-SYDH	12924	0.94	0.80	0.91	
sc584-Rep2-SYDH	4510	0.97	0.90	0.96	
sc585-Rep1-SYDH	8267	0.95	0.86	0.93	
sc585-Rep2-SYDH	2610	0.96	0.89	0.95	
sc585_lotE3113	34333	0.88	0.76	0.78	
sc585_lotF2711	36868	0.88	0.76	0.78	

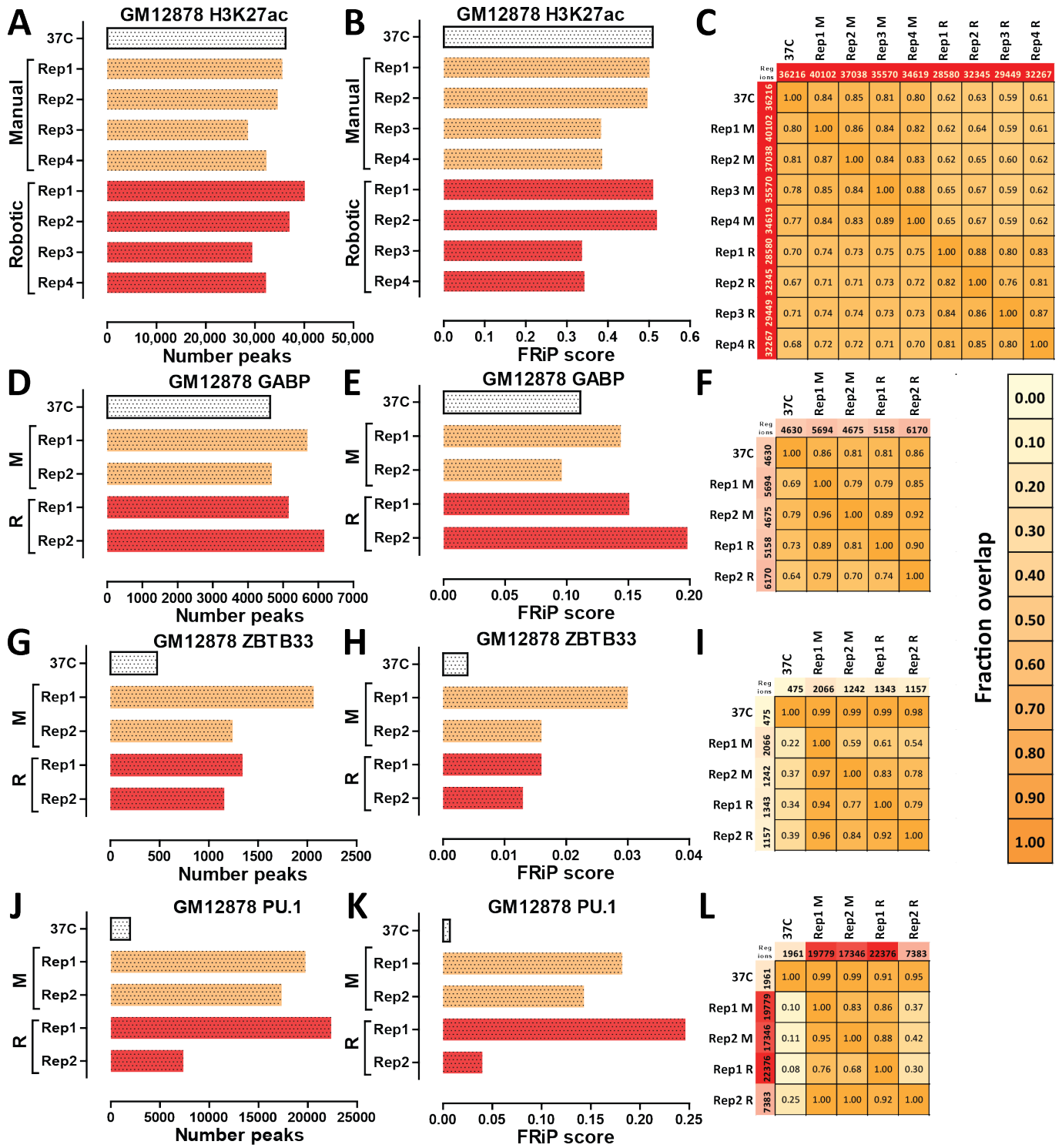
Supplementary Figure 3: Overlap of called p300 peaks with regions of histone mark enrichment in ENCODE data from GM12878 cells. The overlap score (O_{XY}) shown in each box indicates the fraction of peaks in the dataset on the y -axis that are also found in the dataset on the x -axis, i.e. $O_{XY} = |X \cap Y|/|Y|$. The ENCODE histone mark region calls were downloaded from the UCSC Genome Browser.



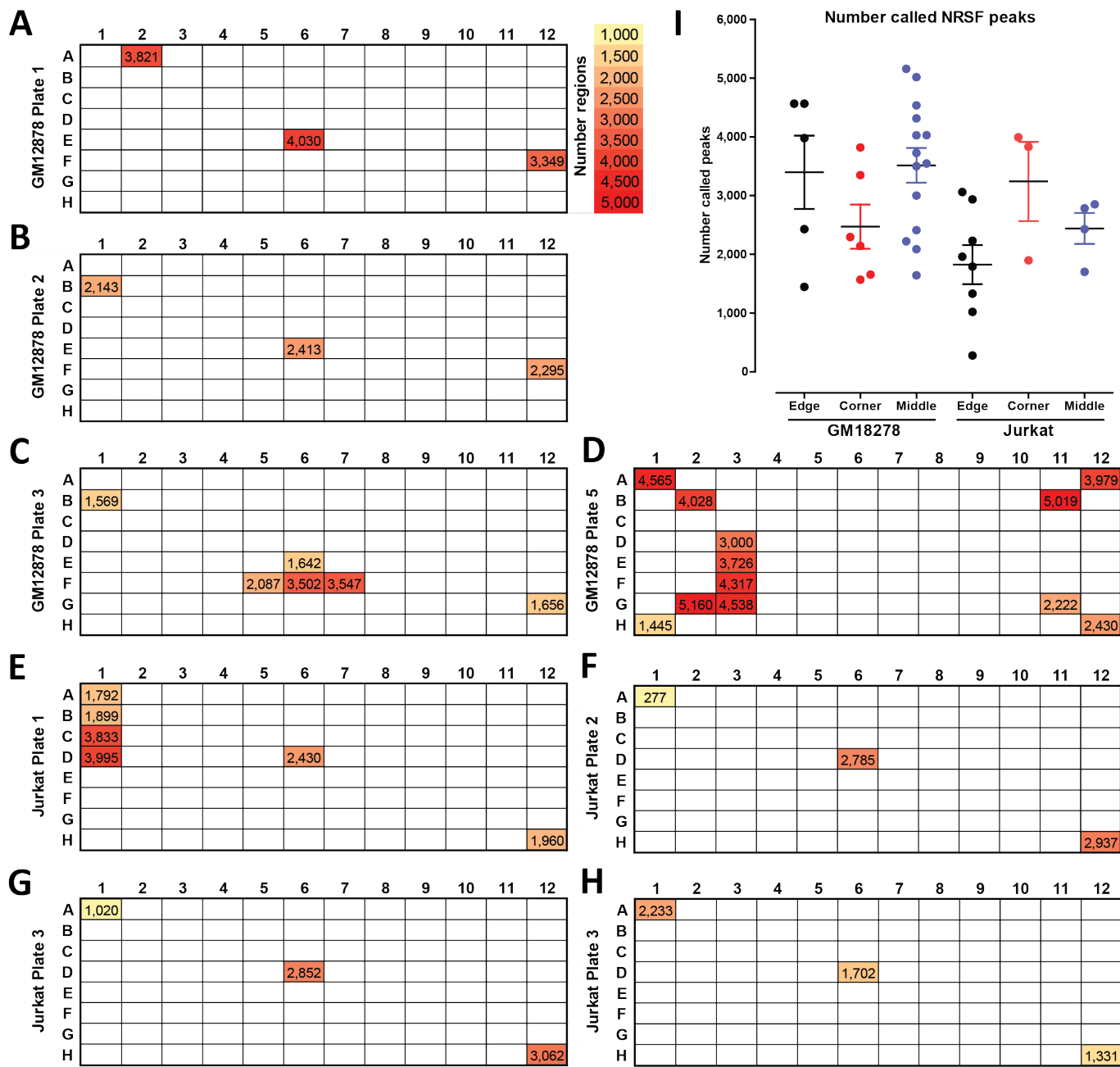
Supplementary Figure 4: Comparison between p300 ChIP-seq results on GM12878 chromatin fixed under standard fixation conditions and chromatin fixed at 37°C. The 1F4-E10P monoclonal antibody was used for all datasets. (A) Number of called peaks; (B) ChIP-enrichment measured by FRiP.



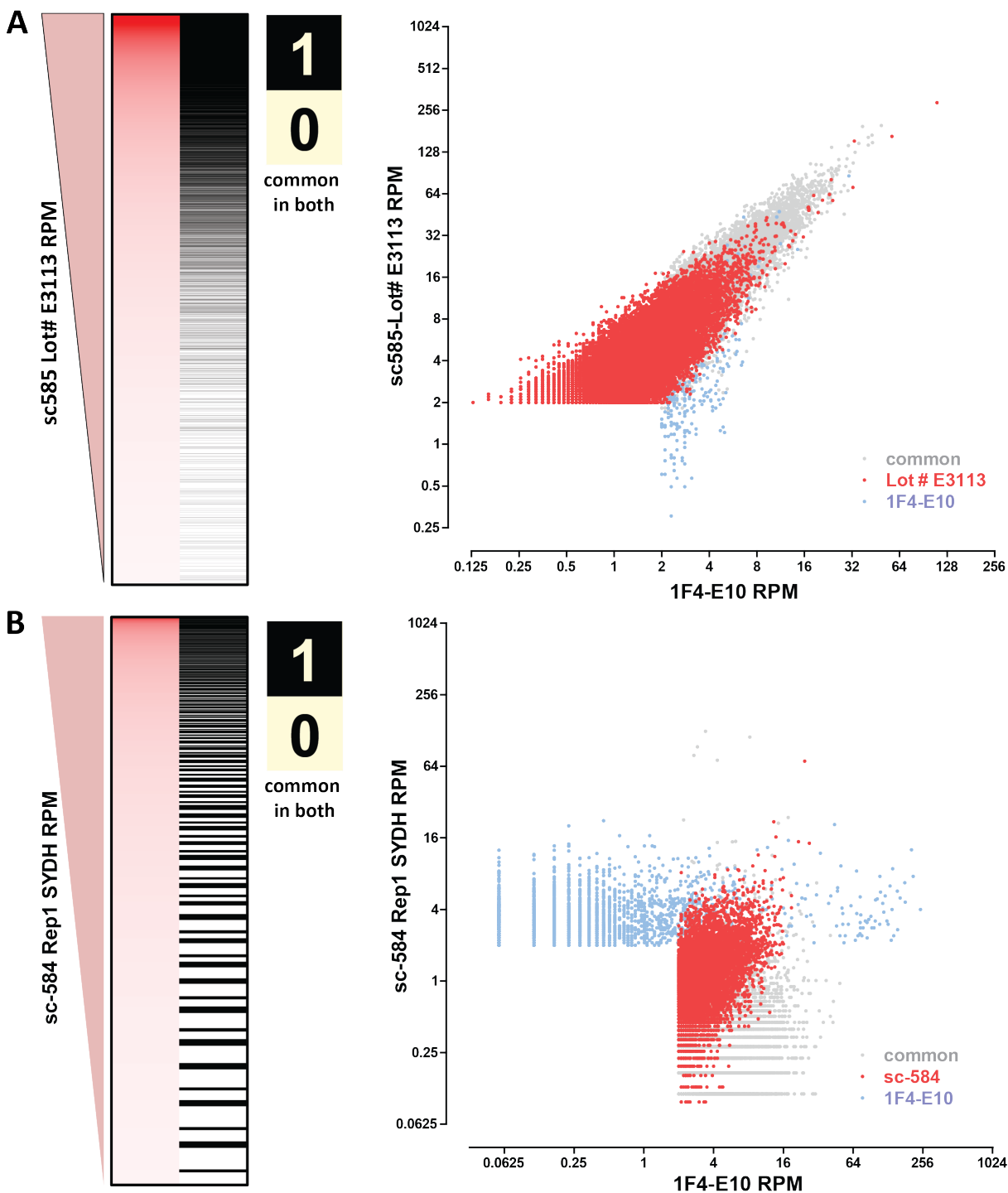
Supplementary Figure 5: Comparison between NRSF ChIP-seq results on GM12878 chromatin fixed under standard fixation conditions and chromatin fixed at 37°C. (A) Number of called regions; (B) ChIP enrichment as measured by FRiP; (C) Overlap between called peaks. The overlap score (O_{XY}) shown in each box indicates the fraction of peaks in the dataset on the y -axis that are also found in the dataset on the x -axis, i.e. $O_{XY} = |X \cap Y|/|Y|$;



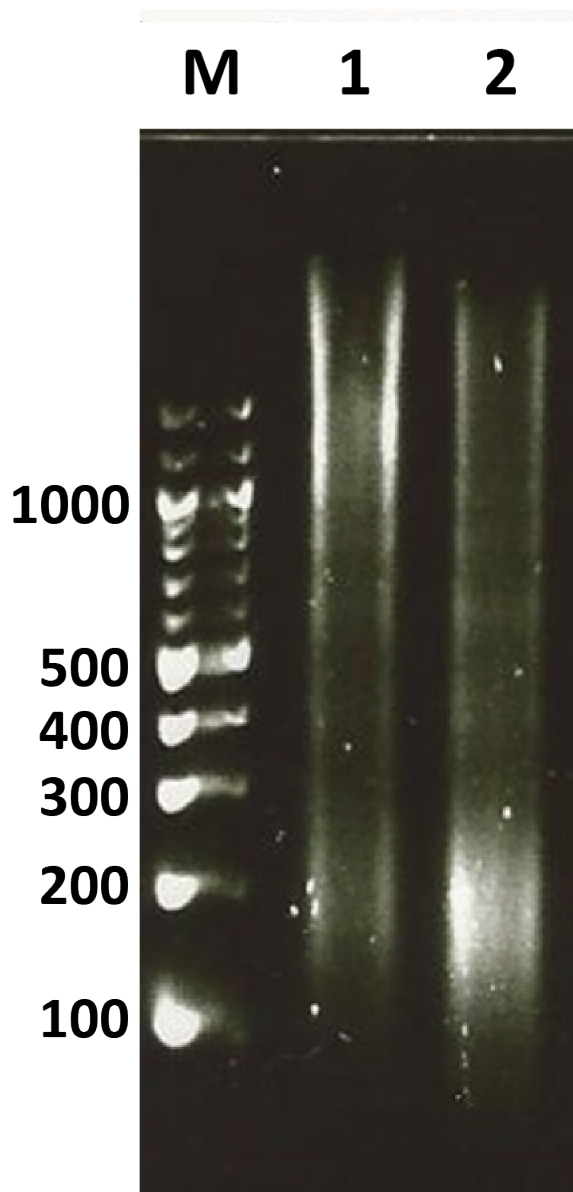
Supplementary Figure 6: Comparison between ChIP-seq results on GM12878 chromatin fixed under standard fixation conditions and chromatin fixed at 37°C for additional targets. (A,B,C) ChIP-seq against H3K27ac in GM12878 cells. (A) FRiP score, (B) number of peaks called, (C) overlap between the sets of peaks; (D,E,F) ChIP-seq against GABP in GM12878 cells. (D) FRiP score, (E) number of peaks called, (F) overlap between the sets of peaks; (G,H,I) ChIP-seq against ZBTB33 in GM12878 cells. (G) FRiP score, (H) number of peaks called, (I) overlap between the sets of peaks; (J,K,L) ChIP-seq against PU.1 in GM12878 cells. (J) FRiP score, (K) number of peaks called, (L) overlap between the sets of peaks. The overlap score (O_{XY}) shown in each box indicates the fraction of peaks in the dataset on the y -axis that are also found in the dataset on the x -axis, i.e. $O_{XY} = |X \cap Y|/|Y|$



Supplementary Figure 7: Examination of the effect of well position on R-ChIP results. (A,B,C,D) GM12878 NRSF R-ChIP plates and the number of called peaks for each dataset with its well position; (E,F,G,H) Jurkat NRSF R-ChIP plates and the number of called peaks for each dataset with its well position. (I) Distribution of the number of called peaks for corner, edge (excluding corners) and middle positions in the plates.



Supplementary Figure 8: Overlap between peaks called with the monoclonal 1F4-E10 and the polyclonal sc-585 and sc-584 antibodies as a function of peak ranking. On the right, the ranking of peak calls in the polyclonal sets according to the RPM (Reads Per Million) values of each peak is shown together with whether a peak overlaps a monoclonal 1F4-E10 peaks (black) or not (white). On the left, the RPMs in each dataset for the union of peak calls between the two are shown. (A) 1F4-E10 (8,430 peaks) vs. sc-585, Lot #E3113 (28,447 peaks); (B) 1F4-E10 vs. previously published by the ENCODE consortium GM12878 p300 ChIP-seq dataset generated using the sc-584 antibody (12,924 peaks)



Supplementary Figure 9: Sonicated GM12878 chromatin used in p300 experiments. A 2% agarose gel (Invitrogen E-Gel), containing samples of fixed chromatin (1% formaldehyde, for 30 minutes at 37°C) sonicated for for 15 minutes (Lane 1) or 30 minutes (Lane2). The sonication volume was 35mL at a concentration of 5×10^7 cells/mL. The final result (Lane 2) was a large batch (sufficient for ≥ 60 ChIP reactions) of sonicated chromatin with the majority of the DNA fragments in the 120-250bp range appropriate for ChIP enrichments that were ultimately sequenced in the standard 50bp single read Illumina format

Supplementary Tables

Supplementary Table 1: Read mapping and dataset quality statistics for robotic NRSF ChIP datasets.
Quality control scores were determined using SPP as described in Landt et al. 2012 and Marinov et al. 2014

Cell Line	Factor	Plate	Well	Library	Complexity	NSC	RSC	QC	Number Peaks	FRiP	Uniquely Mapped reads
GM12878	NRSF	3365	A2	SL26735	0.84	6.165	1.215	1	3,821	0.127	12,246,881
GM12878	NRSF	3365	E6	SL26736	0.86	5.675	1.235	1	4,030	0.144	12,166,760
GM12878	NRSF	3365	F12	SL26737	0.85	3.747	1.143	1	3,349	0.103	16,531,315
GM12878	NRSF	3405	B1	SL28743	0.91	3.808	1.044	1	2,143	0.037	16,286,924
GM12878	NRSF	3405	E6	SL28744	0.89	4.343	1.107	1	2,413	0.048	19,731,464
GM12878	NRSF	3405	F12	SL28745	0.9	3.67	1.092	1	2,295	0.044	19,898,394
GM12878	NRSF	3646	B1	SL34357	0.97	2.541	0.824	0	1,569	0.015	7,567,674
GM12878	NRSF	3646	E6	SL34381	0.97	2.816	0.906	0	1,642	0.017	8,035,350
GM12878	NRSF	3646	F5	SL34380	0.88	3.915	0.811	0	2,087	0.029	4,774,294
GM12878	NRSF	3646	F6	SL34382	0.93	6.315	1.198	1	3,502	0.095	6,378,277
GM12878	NRSF	3646	F7	SL34383	0.93	8.092	1.197	1	3,547	0.095	6,404,751
GM12878	NRSF	3646	G12	SL34384	0.97	1.235	0.815	0	1,656	0.016	6,231,497
GM18278	NRSF	4028	D3	SL46179	0.87	2.729	1.411	1	3,000	0.072	8,672,020
GM18278	NRSF	4028	E3	SL46180	0.66	2.709	1.718	2	3,726	0.096	17,661,548
GM18278	NRSF	4028	A12	SL46217	0.61	4.030	1.262	1	3,979	0.132	15,999,430
GM18278	NRSF	4028	A1	SL46171	0.67	1.444	1.410	1	4,565	0.204	24,180,628
GM18278	NRSF	4028	B11	SL46211	0.51	2.510	1.356	1	5,019	0.200	20,187,765
GM18278	NRSF	4028	B2	SL46173	0.87	2.813	1.128	1	4,028	0.131	10,454,082
GM18278	NRSF	4028	F3	SL46181	0.74	1.461	1.623	2	4,317	0.187	25,430,927
GM18278	NRSF	4028	G11	SL46216	0.89	3.999	1.170	1	2,222	0.040	13,250,634
GM18278	NRSF	4028	G2	SL46176	0.67	1.455	1.907	2	5,160	0.233	21,980,053
GM18278	NRSF	4028	G3	SL46182	0.71	1.479	1.443	1	4,538	0.201	23,259,881
GM18278	NRSF	4028	H12	SL46218	0.85	3.855	1.226	1	2,430	0.052	20,550,794
GM18278	NRSF	4028	H1	SL46172	0.92	2.018	0.975	0	1,445	0.013	24,630,268
GM12878	NRSF	Manual	Manual	ENCODE	0.78	2.121	1.089	1	3,085	0.019	11,945,180
GM12878	NRSF	Manual	Manual	ENCODE	0.89	6.594	2.081	2	3,363	0.070	16,286,742
GM18278	NRSF	Manual	Manual	SL45074	0.89	3.012	1.266	1	3,232	0.105	30,326,354
GM18278	NRSF	Manual	Manual	SL45075	0.94	3.237	1.162	1	2,105	0.039	24,260,996
GM18278	NRSF	Manual	Manual	SL45072	0.90	2.409	1.629	2	3,381	0.068	28,464,754
GM18278	NRSF	Manual	Manual	SL45073	0.87	2.286	1.843	2	3,681	0.078	37,706,507
GM18278 37 °C	NRSF	4028	B10	SL46206	0.88	1.759	1.033	1	2,846	0.076	24,978,799
Jurkat	NRSF	3365	A1	SL26729	0.94	4.948	1.017	1	1,792	0.025	14,447,433
Jurkat	NRSF	3365	B1	SL26732	0.93	4.99	1.061	1	1,899	0.029	17,281,495
Jurkat	NRSF	3365	C1	SL26733	0.78	8.33	1.225	1	3,833	0.144	12,742,979
Jurkat	NRSF	3365	D1	SL26734	0.73	12.468	1.218	1	3,995	0.144	7,551,313
Jurkat	NRSF	3365	D6	SL26730	0.92	8.756	1.141	1	2,430	0.057	13,738,597
Jurkat	NRSF	3365	H12	SL26731	0.92	5.374	1.083	1	1,906	0.029	16,829,422
Jurkat	NRSF	3405	A1	SL28740	0.97	1.075	0.516	0	277	0.001	18,013,669
Jurkat	NRSF	3405	D6	SL28741	0.85	8.854	1.144	1	2,785	0.072	13,132,743
Jurkat	NRSF	3405	H12	SL28742	0.73	8.507	1.141	1	2,937	0.078	14,966,401
Jurkat	NRSF	3435	A1	SL29213	0.96	1.125	0.739	0	1,020	0.006	17,997,043
Jurkat	NRSF	3435	D6	SL29214	0.84	6.077	1.183	1	2,852	0.084	19,801,560
Jurkat	NRSF	3435	H12	SL29215	0.84	6.727	1.189	1	3,062	0.098	18,127,638
Jurkat	NRSF	3549	A1	SL31830	0.7	6.928	1.1	1	2,233	0.045	18,458,174
Jurkat	NRSF	3549	D6	SL31851	0.91	3.993	0.957	0	1,702	0.020	16,754,683
Jurkat	NRSF	3549	H12	SL31882	0.89	1.182	0.852	0	1,331	0.010	19,956,019

Supplementary Table 2: Read mapping and dataset quality statistics for p300 datasets. Quality control scores were determined using SPP as described in Landt et al. 2012 and Marinov et al. 2014

Cell Line	Antibody	Library	Complexity	NSC	RSC	QC	Number Peaks	FRiP	Uniquely Mapped reads
GM12878 37 °C	1A3-F8p	SL31840	0.85	1.244	0.293	-1	3	0	18,894,243
GM12878 37 °C	1F4-E10	SL31838	0.90	1.645	0.422	-1	4,870	0.019	13,446,233
GM12878 37 °C	2E10-D10	SL31839	0.87	1.364	0.269	-1	6	0	13,916,005
GM12878 37 °C	2F4-A8	SL31832	0.85	1.217	0.292	-1	40	0	17,928,468
GM12878 37 °C	2F6-F7	SL31831	0.72	1.541	0.366	-1	9	0	15,450,180
GM12878 37 °C	3B4-G6	SL31835	0.95	1.463	0.266	-1	45	0.001	13,546,541
GM12878 37 °C	3H6-B6	SL31834	0.92	1.242	0.242	-2	5	0	15,393,775
GM12878 37 °C	4C5-A1	SL31833	0.83	1.439	0.287	-1	15	0	12,550,517
GM12878 37 °C	5D2-A1	SL31836	0.89	1.422	0.270	-1	2	0	11,297,381
GM12878 37 °C	5F7-C9	SL31841	0.86	1.365	0.392	-1	2,868	0.010	14,992,412
GM12878 37 °C	7H5-F2	SL31842	0.83	1.447	0.421	-1	1,524	0.051	14,582,010
GM12878 37 °C	1F4-E10	SL34359	0.96	1.836	0.509	0	6,374	0.031	4,802,800
GM12878 37 °C	sc585 lot# E3113	SL34362	0.95	3.467	1.281	1	34,333	0.317	5,811,746
GM12878 37 °C	sc585 lot# F2711	SL34358	0.94	3.659	1.239	1	36,868	0.342	6,688,765
GM12878 37 °C	1F4-E10	SL46203	0.88	1.673	0.852	0	6,333	0.030	20,062,190
GM12878 37 °C	1F4-E10	SL46209	0.87	2.027	0.874	0	6,725	0.030	16,221,052
GM12878 37 °C	sc585 lot# E3113	SL46202	0.88	2.867	1.628	2	28,447	0.257	26,343,063
GM12878 37 °C	sc585 lot# E3113	SL46205	0.92	2.585	1.431	1	11,369	0.066	27,085,241
GM12878 37 °C	sc585 lot# F2711	SL46204	0.93	2.907	1.381	1	15,093	0.091	15,854,599
GM12878 37 °C	1F4-E10	SL45094	0.96	1.798	0.993	0	8,430	0.042	31,243,370
GM12878 37 °C	1F4-E10	SL45095	0.96	1.663	0.978	0	6,181	0.031	33,548,275
GM12878	1F4-E10	SL45092	0.97	1.224	0.580	0	108	0.0004	26,071,005
GM12878	1F4-E10	SL45093	0.97	1.237	0.588	0	252	0.0009	26,022,658
GM12878	1F4-E10	SL46207	0.91	1.146	0.217	-2	37	0.0001	18,794,116
GM12878	1F4-E10	SL46208	0.91	1.152	0.236	-2	17	0.0000	20,417,157
GM12878	sc584	ENCODE	0.92	1.549	0.759	0	12,924	0.063	15,906,721
GM12878	sc584	ENCODE	0.91	1.639	0.765	0	4,510	0.018	16,950,416
GM12878	sc585	ENCODE	0.86	2.088	1.258	1	8,267	0.043	23,366,821
GM12878	sc585	ENCODE	0.88	1.292	0.698	0	2,610	0.011	20,403,419

Supplementary Table 3: Read mapping and dataset quality statistics for H3K27ac, GABP, ZBTB33, PU.1 and input datasets. Quality control scores were determined using SPP as described in Landt et al. 2012 and Marinov et al. 2014

Cell Line	Factor	Rep	R/M	Library	Complexity	NSC	RSC	QC	Number Peaks	FRiP	Uniquely Mapped reads
GM12878 37 °C	Input	Rep1		SL45100	0.96	1.308	0.748	0			25,588,571
GM12878 37 °C	Input	Rep2		SL45101	0.96	1.242	0.613	0			28,422,425
GM12878	Input	Rep3		SL45098	0.96	1.475	0.763	0			21,218,915
GM12878	Input	Rep4		SL45099	0.96	1.381	0.820	0			26,213,457
GM12878	GABP	Rep1	M	SL45068	0.86	2.456	2.343	2	5,694	0.144	28,778,500
GM12878	GABP	Rep2	M	SL45069	0.90	2.615	2.296	2	4,675	0.096	29,626,523
GM12878	H3K27ac	Rep3	M	SL45090	0.89	1.629	1.945	2	35,570	0.502	31,263,444
GM12878	H3K27ac	Rep4	M	SL45090	0.87	1.699	1.811	2	34,619	0.497	36,587,615
GM12878	H3K27ac	Rep1	M	SL45090	0.92	1.463	1.593	2	28,580	0.384	30,476,218
GM12878	H3K27ac	Rep2	M	SL45090	0.93	1.444	1.541	2	32,345	0.386	25,852,868
GM12878	Input	Rep1		SL45096	0.98	1.210	0.683	0			33,254,931
GM12878	Input	Rep2		SL45097	0.98	1.090	0.272	-1			32,985,584
GM12878	PU.1	Rep1	M	SL45076	0.88	6.960	2.143	2	19,779	0.182	27,166,940
GM12878	PU.1	Rep2	M	SL45077	0.89	6.197	2.166	2	17,346	0.143	31,549,754
GM12878	ZBTB33	Rep1	M	SL45080	0.94	1.992	1.250	1	2,066	0.030	22,662,929

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Supplementary Table 3 – *Continued from previous page*

Cell Line	Antibody	Rep	R/M	Library	Complexity	NSC	RSC	QC	Number Peaks	FRiP	Uniquely Mapped reads
GM12878	ZBTB33	Rep2	M	SL45081	0.95	1.614	1.159	1	1,242	0.016	27,577,513
GM12878 37 °C	GABP	Rep1	R	SL46201	0.85	4.153	1.538	2	4,630	0.111	18,416,079
GM12878 37 °C	H3K27ac	Rep1	R	SL46215	0.87	1.542	3.067	2	36,216	0.509	21,994,873
GM12878 37 °C	PU.1	Rep1	R	SL46212	0.89	1.513	0.656		1,961	0.007	22,811,938
GM12878 37 °C	ZBTB33	Rep1	R	SL46213	0.95	1.373	0.376		475	0.004	10,385,335
GM12878	GABP	Rep1	R	SL46174	0.73	3.779	2.651		5,158	0.151	10,708,570
GM12878	GABP	Rep2	R	SL46175	0.63	3.524	3.101		6,170	0.198	13,707,692
GM12878	H3K27ac	Rep3	R	SL46199	0.88	1.590	2.402		40,102	0.511	21,229,446
GM12878	H3K27ac	Rep4	R	SL46200	0.88	1.553	2.801		37,038	0.520	24,739,874
GM12878	H3K27ac	Rep1	R	SL46197	0.92	1.379	1.974		29,449	0.337	20,680,756
GM12878	H3K27ac	Rep2	R	SL46198	0.93	1.358	1.861		32,267	0.343	21,320,237
GM12878	PU.1	Rep1	R	SL46183	0.79	9.692	2.561	2	22,376	0.246	21,431,025
GM12878	PU.1	Rep2	R	SL46184	0.91	2.242	1.561	2	7,383	0.040	23,919,950
GM12878	ZBTB33	Rep1	R	SL46187	0.92	1.891	1.203	1	1,343	0.016	17,536,255
GM12878	ZBTB33	Rep2	R	SL46188	0.92	1.796	1.032	1	1,157	0.013	19,265,589